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ON THE PRACTICAL ASPECTS

OF

THE PREPARATION OF VACCINE VIRUS.

by

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ON THE PRACTICAL ASPECTS

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THE PRODUCTION OF VACCINE VIRUS.

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C. Y. Lin

I. INTRODUCTION.

On account of the high and lasting degree of immunity it imparts to the individual and the almost perfect protection of the community, vaccination against small pox has been adopted, as the first measure in public health work, in almost every country where preventive medicine is practised. It seems, therefore, desirable that during the period of foundation work in preventive medicine in a country like China, where even the elements of sanitation have been sadly neglected, that the line of action should be concentrated first on a disease like smallpox which can be controlled quite effectively by vaccination.

In our experience with several outbreaks of smallpox in Hongkong, we found that the people, as a whole, took to vaccination most readily when once the protection it afforded was made known. The cult of the "conscientious objector" on the part of the people has not been known; this is an indication that the public health situation in China, which in the face of it appears almost insurmountable, presents a ray of hope which is encouraging even to the pessimist. It seems therefore highly desirable that vaccine virus be produced in China on a large scale in order to encourage this phase of public health work.

It may perhaps be of interest first to survey its principal sources of supply in China. Vaccine virus is produced in the following places there:

1. Hongkong (a) Government Bacteriological Institute,
(b) Dr. [illegible]'s Laboratory,
2. Shanghai Shanghai Municipality Health Department Laboratory.

Of the two establishments in Hongkong, the former is only interested in the production of vaccine virus to meet the requirements of the residents within the British possession, and the latter is a private undertaking which supplies the product in various parts of China. The Shanghai Municipality Laboratory is primarily concerned with its production for the supply of the International settlement, but it also sends out its product for sale outside when requested. When offered for sale the prices charged are in all cases considerably high as compared to the actual cost of production. In any case the stock on hand of all these establishments has not been usually sufficient to meet the demand even of its own city in times of serious outbreak of smallpox, and resort has frequently to be made to various Japanese vaccine institutes to supplement local supply.

To familiarise ourselves with the practical points in the preparation of vaccine virus with a view to their possible future application, we have taken for special study this subject. No apology is therefore needed for presenting a paper which is neither illuminating nor contributing to the advancement of our knowledge already acquired on it. It is proposed to consider in general first the various aspects in its preparation, and then to illustrate the actual methods adopted for its propagation by describing in detail that in vogue at the Vaccine Laboratory of the New York City Health Department in this country, and that of the Government Lymph Establishment at London in England, both of which places were visited last summer.

II. HISTORICAL NOTE.

As the first measure in immunology may perhaps rightly be ascribed to vaccination against smallpox which furnishes an excellent illustration of some of the fundamental phenomena of infection, susceptibility and resistance, it may not be out of place here to say a few words on its historical development.

Long before the Christian era the Chinese have learnt the protection to be derived from inoculation against smallpox. This was done by plugging the nostrils with absorbent material soaked in a mixture of the pustular-crustaceous matter taken from the eruption of a smallpox patient, or by blowing the powdered fresh crusts through a small pipe into the nostrils. Owing to the severe reaction set up and its attendant danger, the practice has never gained much popularity.

With the discovery of Jenner in 1796 bovine vaccine virus was introduced, but in those early days it was customary to inoculate human beings with material obtained from the pustules of those previously vaccinated. The great drawbacks to this practice are the inadequacy of the supply upon the appearance of an outbreak, and the possibility of transmitting syphilis and perhaps other diseases. Later on Galbiati began the use of bovine virus in 1803 in Naples and his successor, Negri, introduced its practice in Paris in 1804, and in 1870 its use was first brought into this country.

The great objection then to its universal application was due to its contamination with extraneous organisms, but since Copeman in 1891 showed how to purify it with glycerine, it has come into general use.

In this paper we are primarily concerned with the production of vaccine virus obtained from calves. For descriptive purposes its preparation is best considered under the following main headings:- Seed virus, vaccinifer and vaccine virus.

III. SEED VIRUS.

This refers to the virus used on vaccinating calves or other animals for propagating vaccine virus for human use.

1. Choice of Seed Virus.

Seed virus is one of the most important factors in the preparation of vaccine virus. Experts in various laboratories cannot agree, however, as to which is the best kind of seed virus to use. As a result of this lack of uniformity of opinion among propagators we find quite a variety advocated. In the choice of a seed virus for use we have to learn from the experience of those who have adopted a virus known to be reliable and efficient.

2. Varieties of Seed Virus.

We may call attention here to the following principal kinds of seed virus and their preparations:

a. Human Virus.- This consists of sterilized bone slips charged from serum exuding from ruptured typical vesicles of previously vaccinated children. On the fifth day after vaccination from two to three hundred slips may be charged from each child. The slips are then allowed to dry for a minute, and then placed in a sterilized box and preserved in cold storage where they will remain active for about two to three weeks. This method is difficult to secure at all times and has therefore practically dropped out of use.

b. Glycerinated Bovine Virus.- This is just the same as the virus issued for human vaccination. It is a glycerinated bovine virus which has been ripened for several months and used to vaccinate succeeding calves. It is still very largely used by various vaccine establishments as their only seed virus, although the experience of many investigators indicates that such virus becomes attenuated after a varying number of calf passages.

c. Humanized Virus.- This is a virus derived from people who have been vaccinated with an emulsion of human vaccination crusts. It produces a good vaccine virus, but as it requires the regular collection of human crusts it is not applicable in practice for the average laboratory.

d. Rabbit Seed Virus.- This is obtained by vaccinating rabbits with stock lymph virus and then carrying it on from rabbit to rabbit subsequently. In England this has been used as seed virus exclusively since 1906, and has been found to give excellent results so far.

It is prepared in the following manner: A rabbit with soft white skin, preferably between 3-6 years old, are used for this purpose. An area on its flanks is shaved and washed free from soap. When dry it is scarified with sterile sand paper or match stick and vaccinated by rubbing in the vaccine with a spatula. The vaccine before being applied to the animal is diluted with equal parts of water. The pulp is collected on the third or fourth day. The average yield from each rabbit is 0.4 gram. This pulp is ground into small particles and then diluted with six times by weight of glycerine solution before being stored away in the refrigerator. It is suitable for use after ripening there for a fortnight, but it also keeps potent for several years. Dilute it with equal parts of glycerine-water mixture before use for vaccinating calves. About one cubic centimeter ought to be sufficient for the vaccination of one calf, although the amount varies with the amount of the vaccinated area.

e. Human-Calf-Rabbit Seed Virus.- This is the seed virus used by the New York City Health Department for the propagation of their vaccine virus which is guaranteed to give 100% takes on primary cases of vaccination. The seed virus is obtained from crusts collected from healthy

children about fourteen days after successful vaccination. These crusts are ground up with sterile water into a fine paste and inoculated directly into an area about six inches square upon the abdomen of a calf; the remainder of the animal can be used for vaccinating in the ordinary way. The pulp from this special area is separately collected and treated in the usual manner. After being ripened and tested this bovine virus from human seed is then used in a dilution of one to twelve-and-a-half parts of normal salt solution to vaccinate rabbits. The seed is rubbed well into the freshly shaven skin of the back. Four or five days after, the pulp is removed, ground and diluted in the proportion of one part of pulp to eight parts of glycerine solution. Each rabbit yields about 4 c.c. of this emulsion, and the product from four rabbits is required for the vaccination of one calf in the New York City Vaccine Laboratory.

3. Retrovaccination.

To maintain the efficiency of the virus from generation to generation some form of retrovaccination or reinforcement of the vaccine virus appears to be necessary; some investigators claim that without its employment it is practically impossible to produce vaccine virus whose potency can be relied upon after repeated passage through the same species.

Retrovaccination consists in carrying the vaccine virus back from man to calf or in passing the virus from man through rabbit, monkey or other susceptible animal and then again to calf. This change of species helps to maintain the potency of the virus for an indefinite time, and furthermore, such transfer from one species to another also leaves behind certain associated organisms which may gain increased virulence with successive passage from animal to animal of the same species.

[illegible]. Vaccinifer.

A vaccinifer is an animal employed for the production of vaccine virus. Various animals such as rabbit, donkey, carabao (buffalo), monkey, camel, goat, sheep, pig and other susceptible animals have been used. Most of these are inferior to the calf in the amount of virus or the typical character of the vesicles produced. For our purpose we may consider briefly a few of the more important ones.

1. Choice of Vaccinifer.

Although preference is generally given to the calf, but in case of emergencies other animals may have to be used. In the tropics, especially in the Dutch East Indies and the Philippine Islands, the carabao has been found to yield a more satisfactory virus for use in those regions. It may thus be of interest to consider this question of the choice of vaccinifers.

a. Rabbits.- The value of rabbits as vaccinifers were emphasized by Calmette and Guerin. The virus of the vaccine obtained remains constant for a long period, but the chief objection is the large number of animals to be handled in order to produce a sufficient supply. The rabbit is vaccinated on its back, and the average amount of pulp obtained from each is about 30 centigram only.

Noguchi showed that vaccine virus free from all associated bacteria by means of suitable disinfecting agents, can be propagated in a pure state by vaccinating the testicles of rabbits. The ground up testicular emulsion forms the vaccine virus. Tests carried out by the New York City as well as the State Health Department laboratories indicate that the chief disadvantages of this method are from the standpoint of practicality and the difficulty of producing a virus the potency of which can be depended upon in all cases.

b. Donkey.- According to Brinck and [illegible] the donkey is a very good vaccinifer, especially with young donkey. Ordinary calf vaccine virus is used as seed, and its passage through the donkey increases its virulence considerably. This "asino-vaccine" thus obtained immunizes more rapidly than that of the calf, the immunity being developed in [illegible] days. It can therefore be employed by preference, as the authors claim, during an epidemic and especially upon persons who resist vaccination with the ordinary virus from the calf. The asino-vaccine must be ripened for at least three weeks before use. It attains its maximum activity only when calf vaccine is used as seed virus, but if the asino-vaccine is used instead the potency of the vaccine produced is greatly diminished.

c. Carabao.- In tropical countries one of the great obstacles to successful vaccination in remote places is the difficulty of making available a potent virus, as in many places in the tropics ice cannot be obtained for the transportation and storage of the ordinary vaccine virus. Dr. Wijland of Java devised a method of making vaccine which will retain its potency at ordinary tropical temperature up to 100°F for a period of two months. This vaccine is prepared as follows:

1. Seed virus is humanized vaccine; this is inoculated into calf.
2. Calf vaccine is inoculated into rabbit.
3. Rabbit " " " " calf.
4. Calf " " " " carabao.

The carabao pulp is emulsified with two parts of glycerine and placed at room temperature for four days. After that it is transferred to the refrigerating chamber and kept at -5°C. This emulsion is diluted with two parts of glycerine solution immediately before use.

d. Calf.- For economical and other reasons the calf is generally used in vaccine work. A healthy female calf from two to four months old is preferable. When a young calf is carefully handled, the yield is

more lasting and the resulting eruption is more abundant and runs a more typical course. An older animal may also be used, but the calf obtained from it is not so easily controlled. Besides, with a young calf a milk diet may be used; this avoids the possibility, though slight, of dust contamination from dry feed. For the rest of our paper we shall deal mainly with vaccine propagation on calves.

2. The Use of Calf as Vaccinifer.

Calves for use in vaccination work may be acquired either by purchase or by hire. The former is commonly adopted in this country where the animals after being vaccinated are required by government regulations to be autopsied. The latter is a more economical way of operating and is practised in Great Britain. The animals after use are returned to the contractor who is required to slaughter them and to produce an autopsy report from the government veterinarian.

a. Preparation of Calf.- The calf when acquired is held in quarantine for about a week and is kept under observation by a veterinarian to determine the absence of infection such as tuberculosis, foot-and-mouth disease, [illegible], fever, diarrhoea or skin eruptions of any kind. In some cases it is tuberculin tested, but with a good veterinary inspection this test may be dispensed with.

Separate stables are kept for calves under observation and those under vaccination. The vaccinated calf is held in special stall which is moderately lighted, kept free from dust and screened to keep off insects. The temperature of the animal is taken daily and the progress of each stage of the disease is recorded.

In the [illegible] laboratory the animal is fed on milk, no hay or straw being used for any purpose, since dust of it is [illegible]. However, in the [illegible] laboratory no special attention is paid to [illegible] normal feeding which does not seem to affect the finished product at all.

b. Preparation of Site of Vaccination.- Prior to vaccination the animal is clipped and given a thorough cleansing which includes the feet and tail; this practice, however, is not universally followed. The abdominal wall between the tip of the sternum and the udder including the sides of the thighs and flanks is shaved. Just preceding the vaccination the animal is fastened to the operating table and the site to be vaccinated is thoroughly scrubbed with hot water, green soap and soft brush, followed by sterilized water. The parts are then dried with a sterile towel. The use of germicidal solutions are not desirable for the reason that they are apt to destroy the seed virus.

Authorities differ as to the extent of vaccination permissible on a calf. Some vaccinate a larger and others a smaller area. It is not wise to over-vaccinate as this may result in the production of a vaccine virus of decreased potency.

c. Method of Vaccination.- The usual method is to make long superficial incisions with a sharp scalpel about one centimeter apart in the skin. In order to avoid bleeding the incisions should not be too deep. Seed virus is gently rubbed into these incisions with a spatula. The parts are then allowed to dry and are left entirely uncovered.

As to the best season for vaccination it seems that most parts of the year are suitable for vaccination with except the warm summer months which are rather unfavorable.

The period of incubation varies under different conditions. Generally, within forty-eight hours of vaccination the scratches are pinkish, slightly raised and tender, and within five or six days the vesicles are well developed. The pulp is harvested from the animal about the sixth day. It is advisable to gather it as early as possible in order to avoid contamination infection which may occur from the secondary organisms. Their number after this time may be considerable.

7. Collection.- When the virus is collected the animal is placed upon the operating table. The vaccinated area is thoroughly washed with soap, sterile water and gauze, and wiped dry with sterile gauze. The pulp being ripe is scraped off with a curette and collected in a sterile vessel; the pulp from each animal being put into a separate container. The average output from each calf is about 20-30 grams of pulp. It is desirable that only typical and characteristic vesicles should be gathered. On account of the extremely variable nature of the eruption the decision as to the collection of the vesicles should rest on the judgment and experience of the person in charge. After the pulp has been collected, the animal is killed and then autopsied. If the autopsy shows any signs of tuberculosis or any other disease the virus is discarded. Complete records are kept of each animal and the product obtained from it.

V. VACCINE VIRUS.

Vaccine virus is any substance containing the virus of cowpox, and which has been so treated as to be suitable for use in the active immunization of human beings against smallpox.

1. Difference between Vaccine Pulp and Vaccine Lymph.

It is necessary to note the difference between the two. The pulp consists of the entire vesicle with its contents which is scraped from the skin, and is composed of epithelium, leucocytes, bacteria, products of inflammatory reaction, the fluid contents of the vesicle, fibrin, etc. The lymph is the serous fluid contained in the vesicle or which exudes from the scraped papules. That of the active principle of the virus is [illegible]

a "Vaccinal pulp", and this really is simpler than the lymph alone is used. The pulp which includes the lymph hence contains the virus in greater concentration, which is found exclusively used in this country as vaccine virus. What is termed vaccine virus here is generally known as "lymph", "vaccine lymph" or "calf lymph" in other English speaking countries. From the above definitions it will be apparent that the term "lymph" applied to the emulsion prepared from the pulp is technically a misnomer.

2. Treatment of Material Collected.

The material arising from the vaccination of the calf may be 'treated' in several ways:

A. FRESH VACCINE VIRUS.- This is taken from the vesicles of the calf and used directly for vaccinating human beings. The Institut Vaccinale at [illegible] still practises the use of this kind of virus. At an appointed time the calf duly prepared is taken to the vaccination station. The vesicle is squeezed at its base between the blades of a pair of forceps, and some of the contents is transferred directly from the calf to the arm by means of a flat lancet or any similar instrument. The great objection to this method is the difficulty of having the animals always available ready for use whenever required.

B. GLYCERINATED VACCINE VIRUS.- This consists of the pulp treated with glycerine solution which dilutes and purifies it, and makes it more portable. We shall consider in detail the treatment of glycerinated vaccine virus which is the form now almost universally used.

a. Dilution and purification.- To the pulp collected four parts by weight of glycerine solution may be added, but the amount added is a matter depending upon the consistency and character of the pulp. After weighing the pulp, the dilution should be the proportion of glycerine solution. The pulp is then ground in a special

grinding machine or between the steel rollers of a mixing mill or in a mortar and pestle. The glycerine solution is gradually added and the grinding repeated until the pulp is thoroughly broken up and a uniform suspension obtained.

The glycerine solution is made up as follows: pure glycerine 50%, distilled water 49% and phenol 1%.

Glycerine is added both as a diluent and as a preservative. It destroys the non-spore-bearing bacteria without at the same time seriously impairing the activity of the virus. However, if a higher percentage than 50% of glycerine be added it will soon render the virus inert. Substances other than glycerine are used in addition for the purpose of purifying the virus. Clove oil advocated by Blaxall is used extensively in British Empire laboratories, and carbolic acid first introduced by Kitasato is adopted in Japan as well as in this country.

Freshly prepared vaccine virus is said to be "green". After contact with glycerine in a refrigerator ranging from one to three months, the virus is usually freed from most of the contaminating bacteria and becomes "ripe" for use. The glycerine probably destroys the bacteria by virtue of its dehydrating action. The time required for the virus to ripen depends greatly upon the temperature. Most of the bacteria perish in thirty days at 10° to 20°C. Approximately the same effect may be obtained at 37°C in a few hours. At low temperatures the glycerine has practically no bactericidal effect.

3. Tests against Contamination.- Vaccine virus as it is now prepared always contains bacteria which are for the most part harmless to man, and are largely eliminated by the process of ripening. Nevertheless

may contain from a few thousand to several million bacteria per cubic centimeter. Unusual times contained much fewer and these are mostly harmless saprophytes. The vaccine in [illegible] only upon plating, a year shows that the number of bacteria have been greatly diminished or are entirely absent.

Of the great many diseases which are sometimes attributed to vaccine virus we may perhaps mention tetanus and foot-and-mouth disease. In the U.S. Hygienic Laboratory many hundred samples representing enough vaccine for a million and a half vaccinations have been tested without finding any evidence of tetanus spores. As to foot-and-mouth disease it has been found impossible to convey it to man through cutaneous inoculation.

The following laboratory tests may be made on the finished product as a precautionary measure:

1. Plate a loop of the vaccine upon agar, incubate overnight and count the colonies. This is repeated at regular intervals.
2. Put five drops of the vaccine into glucose broth in fermentation tubes and incubate for evidence of gas forming organisms.
3. If the fermentation tube culture shows gas formation within nine days inject guinea pig with culture and observe for two days for evidences of tetanus.
4. Inoculate a guinea pig subcutaneously with 0.25 c.c. of the freshly prepared vaccine and watch for ten days for signs of streptococcus infection.

8. Storage and Effect of Temperature.- The vaccine virus from each animal is put separately in convenient sized bottles and kept in cold storage at a temperature of 4° to 5°C. Vaccine which has been put up in packages ready for issue may be kept in an ice-box at a temperature of 35° to 40°F. The life of the vaccine or the length of time between its collection and its use varies largely according to the temperature it is stored.

Elgin showed that vaccine virus exposed to a low temperature in a cold storage will remain active for a long time without deteriora-

tion, that stored in an ice-chest at a temperature below [illegible] it deteriorates slowly, and when exposed to room temperature especially in the summer months it rapidly loses its potency. The Local Government Board sent their glycerinated vaccine virus, kept in cold storage, on trips around the world for a period of two years and after its return to England the virus was still found to be potent. This confirms the observations noted as to the keeping qualities under low temperature.

d. Standardization and Potency Tests.- Several methods are employed for determining the efficacy and potency of vaccine virus before distributing it for general use. The accepted methods agree in using animals before permitting the virus to be used on man. Theobald Smith recommends the skin of the calf and requires that the eruptions produced there must correspond with those of the skin of a child. Calmette and Guerin as well as Camus employ the skin of the rabbit; while Chaumier and others first employ animals, and then observe the results on a few children before issuing the virus. There is agreement among the authorities that the skin of the rabbit and calf are reliable indications of the effects produced on human subjects. Henseval and Convent believe that a preparation which produces on the skin of a rabbit almost confluent eruptions in a dilution of 1 to 500 should be considered a suitable virus. [illegible] uses a vaccine of known potency as control, and both the test and the control vaccines in a dilution of 1 to 1000 are used to vaccinate a rabbit and the results compared. According to [illegible] the ultimate criterion of the potency of this agent is the percentage of "takes" in primary cases of vaccination.

In the New York City Health Department the practice is to make [illegible] inoculations of the [illegible] vaccine virus [illegible] previously [illegible]

cinated children. These must all show a perfect take before the vaccine is passed as up to standard.

4. Distribution.- After having passed the necessary tests the vaccine is put up for issue in special containers, such as glass vials or in capillary tubes.

The capillary tubes are filled either by hand or by the vacuum method. The latter is the more efficient and is also a government requirement in this country. As success with the vacuum method depends upon careful manipulation we may give the method in use at the New York City laboratory in detail:

> The capillary tubes sealed at one end are placed with the sealed ends uppermost in a cylindrical container together with a glass tube 3/16 inch in calibre open at both ends. This glass tube is placed in the centre of the capillary tubes and is used for introducing the virus.
> 2.5 c.c. of vaccine virus to every 100 capillary tubes is the usual ratio. After introducing the virus the cylindrical glass container is placed in a vacuum apparatus. The air is carefully exhausted until a vacuum of 18 inches is produced. By allowing the air to re-enter very slowly the virus rises to the desired position, usually above the centre, and is retained in the capillary tubes.
> A disc of wire gauze of No.20 or No.30 mesh is placed in the lower part of the container to prevent the ends of the capillary tubes from sticking to the bottom of the container.
> To avoid vaccine virus running up the outside of the capillary tubes they should not be packed in too tightly.
> The loss by imperfect filling of tubes with this method is about 5%.

These capillary tubes are put up in packages on which are marked the date of issue or the date of expiration. In Europe it is not customary to date vaccine virus, but in the United States it is required to be placed on each package. Manufacturers have generally made their products not reliable after a certain date or three months after the date of issue. The virus should remain active for at least three months provided it is kept during the whole of that period at a temperature of 32° to 40° in the ice-box.

C. DRIED VACCINE LYMPH.- In order to secure a vaccine with good keeping qualities and capable of transportation for long distances, the vaccinal matter may be dried, and in this dry state it will remain potent for a very long time. Dried vaccine has been found to be able to withstand high temperature without much attenuation of its virulence. It is thus a valuable form of vaccine for use under unfavorable conditions of transportation and climate. In preparing the dried vaccine the process of desiccation must be done without the application of heat and must be as rapid and complete as possible. There are several methods recommended for its preparation.

Schmid uses the pulp obtained in the ordinary way by grinding it up in a sterile mortar and spreading it over a large surface under aseptic conditions. After that it is dried rapidly over a hygroscopic chemical in vacuum.

Degive recommends drying the ground up pulp in a drying chamber by passing through a current of pure air for three hours, the temperature of the air not to exceed 25°C.

According to Otto the ground-up pulp is first frozen and while in this state it is submitted to constant vacuum in the presence of sulphuric acid or phosphoric acid.

The dried vaccine thus obtained is pulverized in the drying chamber, after which it is introduced into brown glass tubes and sealed by fusion under a maximum vacuum. In these tubes it should be stored away in the cold protected from heat and light, particularly from sunshine. They are packed up separately in wooden boxes and sent out to the distributors without having to take the precaution of keeping them at lower temperature as is the case with glycerinated vaccine lymph. When opened the dried vaccine should at once be emulsified with four parts by weight of glycerinated water and kept cold for use.

VI. VACCINE ESTABLISHMENTS.

Vaccine establishments vary greatly both in their equipment and in their operation, and I shall touch on these only in a very general way.

1. Equipment.

The buildings consist of operating room, laboratory, cold storage, animal rooms and stables. The operating room generally has white-tiled walls and concrete floor and is usually equal to that of an ordinary hospital. It is equipped with special operating tables which may be of the ordinary tilting variety, or it may be that of the [illegible] kind. The laboratory is of the standard type and needs no special mention here. In some special methods for manipulating the pulp is adopted it may have the particular grinding machines. The stalls are divided into quarantine stables and incubation stables. In the former animals are kept under observation, and in the latter inoculated animals. The latter should be so constructed that the floors may be flushed daily with water to wash away the dejecta of the animals.

2. Operation.

In this country vaccine establishments are, with few exceptions, conducted by private enterprise, whereas in European countries they are generally operated by the government. The advantages of state control are well shown as in the case of Germany, where there are twenty-two public laboratories. Their data each year are consolidated and published annually in the "Medizinal-statistische Mitteilungen aus dem Kaiserlichen Gesundheitsamte". This general report describes the individual laboratories and gives the cost of maintenance as well as the number of calves. It tells what is the method used in caring for and inoculating the animals, collecting the virus, the production, [illegible] of preserving and distributing the virus, indicates all experiments and scientific observations made during the year in each laboratory. [illegible]

VII. METHOD OF PRODUCTION OF VACCINE VIRUS BY THE NEW YORK CITY HEALTH DEPARTMENT.

The Vaccine Laboratory of this city Health Department is located at Otisville, N.Y. Virus [illegible] [illegible] [illegible] [illegible] [illegible] collected is sent to the city laboratory for treatment there.

Usually calves from three to six months old are preferred. The animal is kept in quarantine for a week and also tuberculin tested before use. After having passed the veterinary observation, it is clipped and washed and the hoofs scrubbed as well.

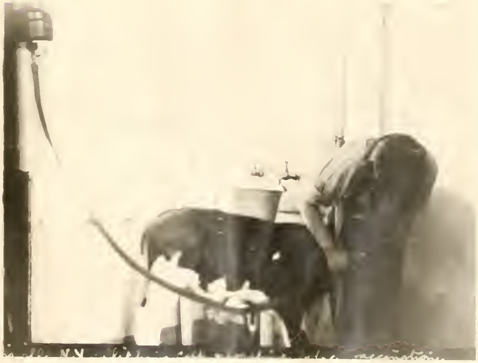

Fig. 1.- Clipping and Washing of Calf.

The animal is then placed on a bench on its side with the hind leg fastened in vertical position, and the site to be vaccinated comprising the abdominal wall and the inside of the thighs is shaved and washed (Fig. 2). It is then taken to the operating room where it is fixed on to the operating table shown in Fig. 3. The site of vaccination is washed with sterile water followed by alcohol. When the skin is thoroughly dry a series of superficial linear

incisions is made with a scalpel about one-fourth of an inch apart. On this incised area is rubbed in the seed virus which [illegible] [illegible] has been already referred to. The vaccination is done in sections until the whole field is covered. Some time is allowed for the vaccine to dry before the animal is released and taken to the stanchion (Fig. [illegible]).

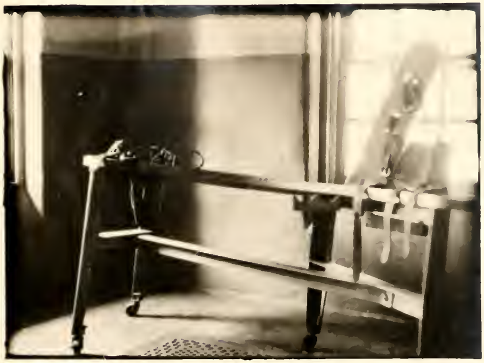

Fig. 8.- Operating Table of Cast Iron.

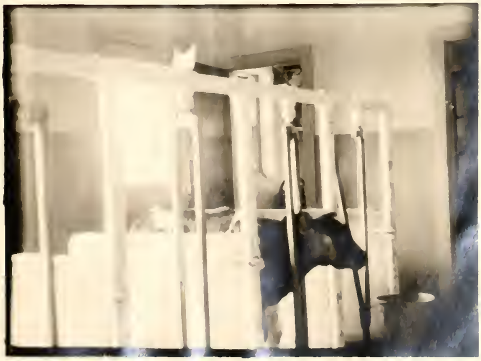

Fig. [illegible] [illegible] Stalls.

The animal is held aside for [illegible] [illegible] [illegible] [illegible] from [illegible]. The temperature and general condition is observed daily. On the sixth day the pulp is collected. For this purpose the calf is again placed on the table in the operation room. The inoculated area is flushed with sterile water and dried. The vesicles are removed with a sharp curette scraping only once over each part in order not to have any blood mixed up with the pulp.

Fig. 4.- Showing Extent of Inoculated Area after Removal of Pulp.

The material collected is weighed and emulsified by rubbing it with a solution containing glycerine 50%, carbolic acid 1% and water 49%. The proportion of pulp to the solution is one to four. The emulsion is passed through a [illegible] glass [illegible] after having been ground up with mortar and pestle. It is then taken through a 100-mesh wire gauze strainer. The resulting [illegible] [illegible] is bottled in 100 c.c. and kept in cold storage at -10° to -15° [illegible] [illegible] [illegible].

A bacteriological examination is made of the vaccine which is also tested for [illegible] with [illegible] [illegible] and [illegible]. The bacteriological details of which need not be given here, as the laboratory technique [illegible]

factory [illegible] it has tested clinically on many cases. The vaccines are examined at each reading [illegible] inoculations, all the fifteen inoculations must take correctly before the vaccine is issued for general use. Every lot which on the second [illegible] tested clinically and [illegible] the take is proved to fail and the vaccine is destroyed. [illegible] the expiration date is [illegible] kept [illegible].

The special feature of this laboratory in preparing its product is the use of the human-calf-rabbit seed as well as the very close linear incisions made on the calf in order to get confluent eruptions.

XIII. METHOD OF PREPARATION OF VACCINE VIRUS AT THE BRITISH GOVERNMENT LYMPH ESTABLISHMENT.

This establishment, the biggest of its kind as it prepares vaccine for the supply of the whole country, is located at Hendon, a suburb of London. The buildings consist of a magnificent modern laboratory, cold storage plant, an animal house and four stables.

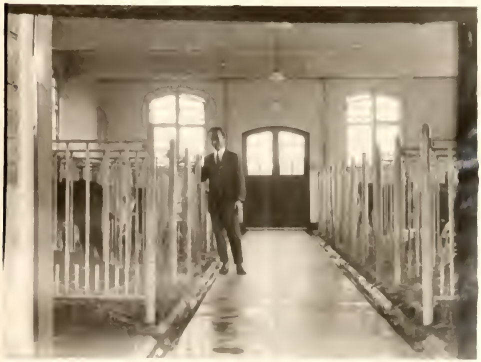

Fig. 1. Stable for Calves.

The calves used here are obtained from the dairy section through a contractor. They are from four to six months old, and must be perfectly healthy and have a good clean skin. They are observed under quarantine for a week before used. The day previous to vaccination the temperature of the calf is taken; this should be about 102°F. The animal is then clipped, shaved, and washed.

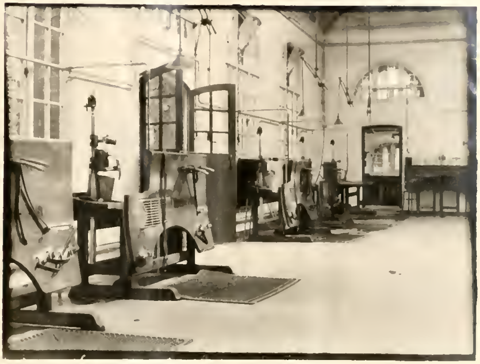

Fig. 6.—Operating Room Equipped with Tilting Operating Tables.

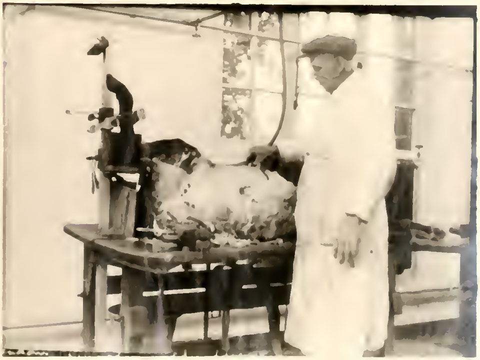

Fig. 7.—Shaving and Washing of Calf.

[illegible] [illegible] [illegible] [illegible] (Fig. 7). The method of [illegible] [illegible] [illegible] [illegible] is shown in Fig. 8. [illegible] applied [illegible] [illegible] [illegible] (Fig. 9).

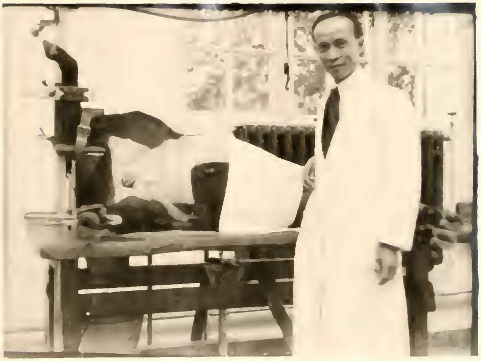

Fig. 8.- [illegible] [illegible] of [illegible] [illegible].

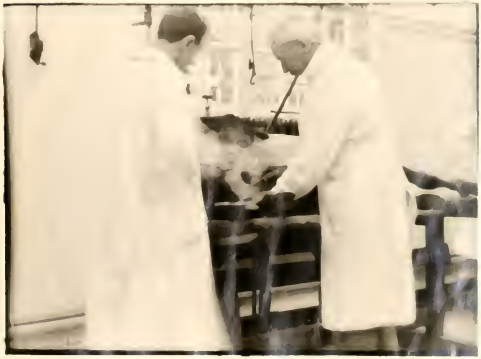

Fig. 9.- [illegible] [illegible] [illegible] [illegible] [illegible] [illegible].

[illegible] The tem- perature is taken on the [illegible] day, and it is generally between [illegible]. On the fifth day the vesicles are collected. Great care is exercised in the classification of the development of the vesicles before collection.

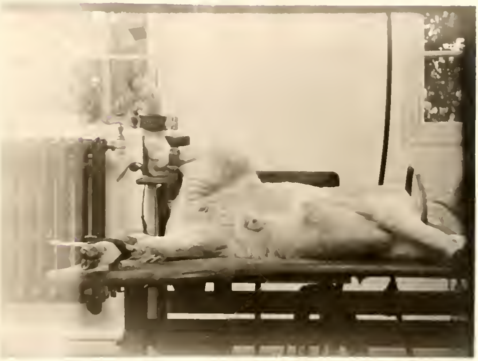

Fig. 12.- Showing Nature of Vesicles on Fifth Day after Vaccination.

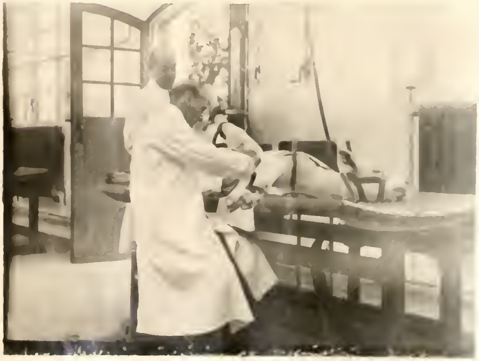

Fig. 13.- Collecting [illegible] from Vaccinated Calf.

[illegible]

[illegible]

[illegible]

[illegible]

The vaccine is finally tested and if the results are fully satisfactory, is filled into small capillary tubes, each holding enough [illegible] sufficient for one vaccination. The tubes are sealed individually with a small hand machine (Fig. 13).

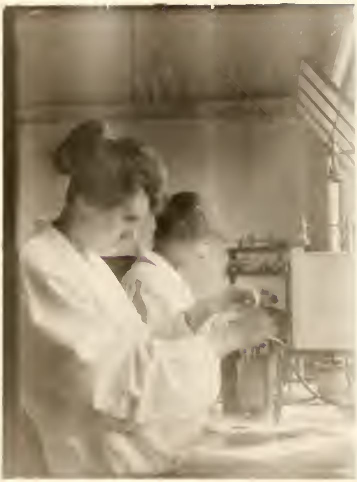

Fig. 13.— Charging Capillary Tubes with Hand Machine.

Certificates [illegible] are collected and the results [illegible] in a register together with the results of all the cases vaccinated with the product from each animal. These reports for the year [illegible] to a total of nearly three million primary cases vaccinated with this product, show a success of 99.4%.

The methods in use in this laboratory and regarded as essential to the successful production of vaccine virus consist in the avoidance of over-ripening, the use of [illegible], and the [illegible] of the [illegible] according to the quality of the vesicles developed on the calf.

IX. CONCLUSION.

In studying vaccine production, although certain features of the work are practically common to all laboratories visited, yet each laboratory has peculiarities and special features about the equipment of which it is claimed to be well [illegible] possible to produce reliable vaccine virus. This would seem to indicate that although certain [illegible] principles are accepted yet the question of producing vaccine under standard conditions is still to be solved. Our knowledge upon the subject so far is not sufficient to admit of any definite standardization as to the methods of preparation and potency tests.

X. ACKNOWLEDGMENT.

We are glad to say that our reception was especially cordial and that every advantage was extended at the places visited. Our thanks are due [illegible], Director of the [illegible] Vaccine Laboratory at Marseilles, and to Dr. T. [illegible] of the [illegible] Lymph Laboratories [illegible] for placing at our disposal all the facilities of their laboratories [illegible] results and other data [illegible] the preparation of their vaccine, as well as the [illegible] extended to us [illegible] during the course of our stay at their laboratories.

XI. REFERENCES.

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